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(71) Applicant: HYBRITECH INCORPORATED [US/US]; 11085 Torreyana Road, San Diego, CA 92121 (US).

(72) Inventors: BARTHOLOMEW, Richard, M. ; 13431 Sundance, San Diego, CA 92129 (US). BEIDLER, Daniel, E. ; 304 Glencrest Drive, Solana Beach, CA 92075 (US). DAVID, Gary, S. ; 9477 Poole Street, La Jolla, CA 92307 (US).

(74) Agents: RESPESS, William, L. et al.; Lyon & Lyon, 611 West Sixth Street, 34th Floor, Los Angeles, CA 90017 (US).

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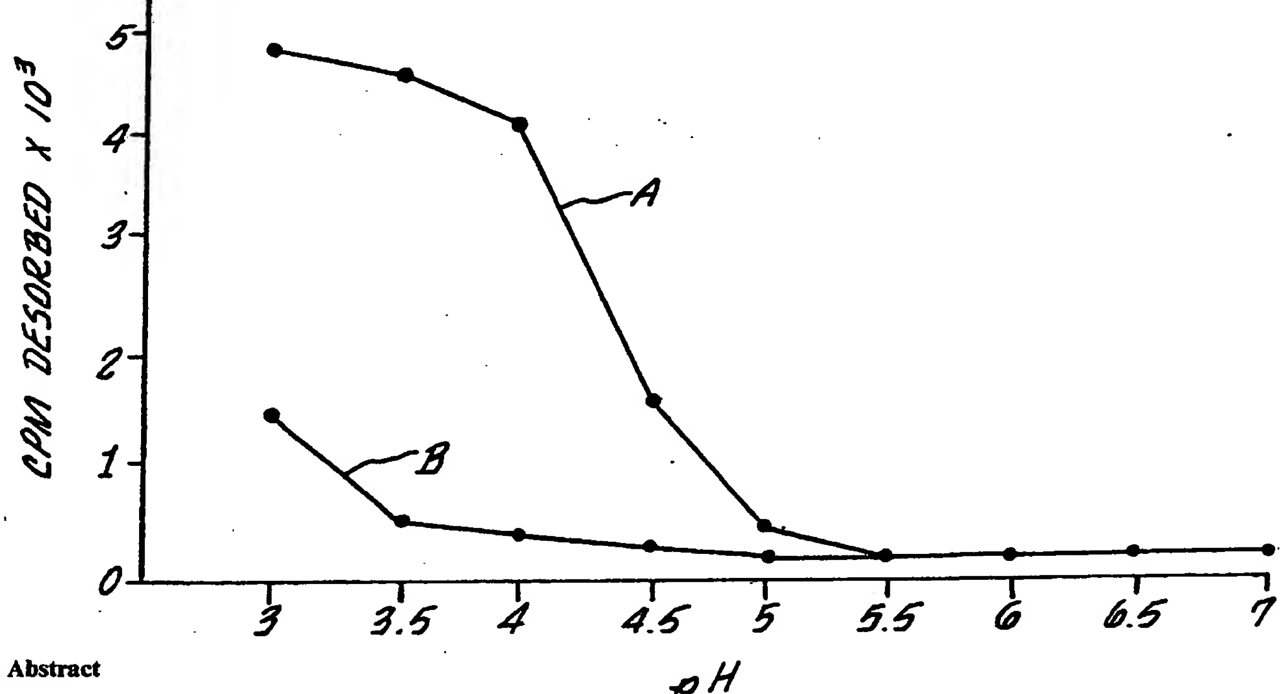

(57) Abstract

Process for affinity purification of antigens and antibodies that employs monoclonal antibodies having a high affinity for the antigen in a first environment and a low affinity in a second environment, the environments being ones in which the immuno-ch mical properties of the antigens and antibodies are not adversely affected. Also described is a process for fractionating antisera to obtain an antibody fraction having similar antigen binding properties as exhibited by the environmentally sensitive monoclonal antibodies. The effect of varying pH in the binding capability of tow monoclonal antibod-

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DESCRIPTION

METHOD OF AFFINITY PURIFICATION EMPLOYING MONOCLONAL ANTIBODIES

Field of the Invention

This invention relates to the purification of antigens and antibodies by affinity chromatography. In another aspect it relates to monoclonal antibodies.

Background

The purification of an antigen by affinity chromatography using serum antibodies, produced by a host animal's response to the antigen, that are bound to a solid support as an immunoadsorbent is a process which has been used for many years. This process has, however, at least two serious shortcomings which impair its usefulness. Thus, if antibodies of high affinity are used to extract the antigen from a sample, harsh conditions are required to dissociate the antigen from the antibodies after non-absorbed impurities have been washed from the body of immunoadsorbent. The conditions required for this, for example, a pH of less than 3 or greater than 11 or a concentrated chaotrope such as guanidine or urea solution, can denature the antigen and the antibodies, diminishing, if not destroying, the immunochemical and/or biological properties of the antigen and shortening the useful life of the immunoadsorbent.

To avoid the problems associated with the use of antibodies having a high affinity for the antigen, it has become common practice to use immobilized antibodies of low affinity as an immunoadsorbent. Use of these antibodies permits elution of the antigen from the body of immunoadsorbent using mild, non-denaturing conditions. However, the requisite step of washing the column to elute impurities from the bound antigen also elutes some of the

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antigen, so much so that the efficiency of separation is greatly reduced. In addition, low affinity antibodies cannot efficiently bind antigens which are present in the media at relatively low concentrations, i.e., less than about 10 ng/ml.

With the advent of hybridoma technology, it has become possible to obtain monoclonal antibodies, which subsequently have been proposed for use as immunoadsorbents in the affinity purification of the antigens against which they were raised. See, for example, Stenman et al, J. Immunological Methods, 46, 337 (1981); Stallcup et al, J. Immunology, 127, 924 (1981) and Katzman et al, Proc. Natl. Acad. Sci. USA, 78, 162 (1981). These reports suggest that the monoclonal antibodies employed, at best, had only a modest affinity for the antigens, permitting their desorption from the immunoadsorbent using mild conditions. Thus, the experience to date using monoclonal antibodies as immunoadsorbents suggest that their properties should parallel those of the "polyclonal" antibodies of conventional antisera, i.e., the use of low affinity antibody permits elution of the antigen under mild conditions whereas use of a high affinity antibody requires harsh conditions to dissociate the antigen from the antibody.

Summary of Invention

As is well known, hybridomas are formed by the random fusion of B-lymphocytes with myeloma cells in the presence of a fusion promoting agent. Each hybridoma of the large population of hybridomas which can be produced by a fusion secretes a different monoclonal antibody. Typically, the population of hybridomas is screened to select for further cloning those that secrete an antibody of the desired antigenic specificity in order to obtain useful quantities of antibody. We have found that, among the population of hybridomas which secrete antibodies against a specific antigen and the subpopulation of those which secrete

antibodies having a high affinity for the antigen, a very much smaller population secretes antibodies which have a high affinity for the antigen in a first environment, but a much lower affinity in a second environment, neither environment being detrimental to the immunochemical or biological properties of either the antigen or antibodies. We believe that the existence of these antibodies in high affinity antisera has gone unrecognized because the majority of the high affinity antibodies in the antisera are ones which require harsh conditions before the antigen can be separated from the antibodies and they dominate the immunochemical properties of the antisera.

Accordingly, we have found that we can screen a population of hybridomas, which can be the product of multiple fusions, and identify those which produce a monoclonal antibody having a high affinity in a first environment and a low affinity in a second environment and clone at least one of the hybridomas to obtain a sufficient quantity of the antibody it produces to permit its use as a highly effective immunoadsorbent for affinity chromatography. As used herein, an antibody is considered to exhibit a high affinity when its affinity constant (Ka) is about $\geq 10^9$ and to exhibit a low affinity when its Ka is about $\lesssim 10^8$.

Brief Description of the Drawings

Figures 1 and 2 are graphs of data reflecting the effect of changes in pH on the desorption of radiolabeled human growth hormone bound to four different monoclonal antibodies immobilized on a solid phase.

Description of Preferred Embodiments

According to our invention, purification of an antigen is accomplished by a process comprising the steps:

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a) selecting a monoclonal antibody having a high affinity for the antigen in a first environment and a low affinity for the antibody in a second environment, neither environment causing substantial, irreversible changes in the desired immunochemical properties of the antigen;

b) immobilizing the antibody on a solid support;

c) contacting the immobilized antibody with a sample containing impure antigen in the first environment to bind the antigen to the antibody;

d) separating unbound impurities from the bound antigen; and

e) eluting the antigen in a purified form from the immobilized antibody using, as an eluant, a medium which is the second environment.

As already noted, antibodies useful in our invention can be obtained by screening the antibodies produc d by a population of hybridomas obtained by the fusion, using known methods, of myeloma cells with B-lymphocytes. The B-lymphocytes are typically spleen cells taken from a hyperimmunized animal to which the target antigen has previously been administered as an immunogen. After those hybridomas that produce monoclonal antibodies whose specificities are against the desired antigen have been identified, they can be further screened to identify those that produce antibodies whose affinities vary with changes in environment which are not damaging to the antigen or antibody. For example, antigens are usually stable in solution within the pH range of 4-10.5. To obtain a pH sensitive antibody for use as an immunoadsorbent, the population of monoclonal antibodies is screened to id n-tify those which have a high affinity for the antibody at one pH within the range, i.e., a Ka of about 10^9 and preferably $\geq 10^{10}$ and a low affinity at a second pH,

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i. ., a Ka of about 10^8 and preferably less than 10^6 within the same range. This kind of screening can be done by immobilizing the antibody on a solid support and, after permitting it to bind antigen, measuring the extent of desorption of the antigen that occurs at different pH levels, a measurement which can be made, for instance, by employing radiolabeled antigen and counting the radiation emitted by the solid phase and/or supernatant. A similar screen can be carried out to identify antibodies that respond to other kinds of environmental change.

It is presently preferred to exploit monoclonal antibodies whose capability to bind antigen is sensitive to changes in pH. In this regard, the antibody is selected to have a high affinity at one pH and a low affinity at a second pH which may be higher or lower than the first pH. Usually the first pH will be at or n ar pH 7 although it need not be. However, it is also within the scope of the invention to select antibodies which respond to a different kind of change in environmental condition. For example, a monoclonal antibody can be selected which undergoes a change from high low affinity in the presence of a chaotropic solution as the eluting medium. Among suitable chaotropes are KBr, KI, KSCN, guanidine, urea and $MgCl_2$. Thus, monoclonal antibodies can be selected having a Ka $\geq 10^9$ in the absence of chaotrope but which has a Ka of $\leq 10^8$ in the presence of the particular chaotrope whose concentration is not detrimented to the antigen in question. The selection for chaotrope sensitivity can also be made in buffers at a specific pH. Alternatively, antibodies can be selected which are sensitive to changes in pH in the presence of a constant concentration of a chaotrope.

Monoclonal antibodies whose affinity for an antigen is adequately lowered by changes other than pH or concentration of chaotrope can also be selected. Among the kinds of media sensitivity for which the antibodies can be

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screened to select those whose antigen binding ability is affected by a change in eluting medium can include borate sensitivity, methylmannoside sensitivity and sensitivity to non-ionic or ionic detergents and reagents which affect specific amino acids such as tryptophan and tyrosine.

For use in affinity chromatrography, a selected monoclonal antibody can be bound to any of the solid supports commonly used in affinity chromatography. These include sepharose, polystyrene, glass, nylon, cellulose, polymethyl methacrylate, silicagel, polyacrylamide and nitrocellulose.

The following examples illustrate the application of the present invention to obtaining monoclonal antibodies whose binding affinity for an antigen varies from a high affinity in a first environment to a low affinity in a second environment, neither environment causing damage to the immunochemical properties of the antigen and their usefulness as immunoadsorbents for affinity chromatography.

Example 1

Spleen cells taken from Balb/c mice hyperimmunized with human growth hormone (HGH) were fused using polyethylene glycol with mouse myeloma cells (NS-1 or SP-2/0 lines). The resulting hybridomas were cloned and screened to determine those secreting antibody specific for HGH by a radioimmunoassay employing ^{125}I-HGH and horse anti-mouse IgG on sepharose beads. The hybridomas producing anti-HGH were further screened to identify those producing antibodies having a Ka of at least about 10^9 at pH 7. These were further screened to identify those whose affinities were sensitive to changes in pH over the range from 4 to 10.5. Data reflecting the pH sensitivity of four monoclonal antibodies is shown in Tables 1 and 2 and Figs. 1 and 2. The individual antibodies are designated by the letters A, B, C and D, respectively. These data were

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obtained in the following way: HGH labeled with ^{125}I was bound at pH 7 to each antibody which had previously been immobilized on polystyrene balls. Each ball contained approximately 1 ng of antigen and 10,000 cpm. Three of each were incubated in 1 ml of PBS in 10% horse serum for four hours at the pH indicated. The adjustments in pH were made by the addition of either a buffer of sodium carbonate (10% in horse serum) to obtain pH 7 or by the addition of sodium acetate buffer (10% in horse serum) to obtain pH 7. After incubation, 800 μl of the supernatant was counted. The counts of desorbed antigen at each pH are recorded in Tables 1 and 2 and plotted in Figs. 1 and 2.

Table 1

Counts/Minute x 10^{-3} of Desorbed HGH*

pH	Antibody A	Antibody B
3.0	4.810	2.442
3.5	4.625	0.803
4.0	4.156	0.357
4.5	1.608	0.248
5.0	0.449	0.206
5.5	0.176	0.162
6.0	0.220	0.176
6.5	0.336	0.167
7.0	0.328	0.162

* Average of three supernatants

Table 2

Counts/Minute x 10^{-3} of Desorbed HGH*

pH	Antibody C	Antibody D
7.0	0.236	0.187
7.5	0.208	0.336
8.0	0.240	0.321
8.5	0.666	0.277
9.0	0.401	0.237
9.5	1.038	0.287
10.0	3.030	0.364
10.5	4.809	0.584
11.0	5.508	3.460

* Average of three supernatants

The data in Table 1, particularly as plotted in Fig. 1, show that the binding of HGH by antibody B was essentially insensitive to changes of pH over the range pH 3.5-7 but that the binding of HGH to antibody A was significantly decreased in the range pH 4.5-4.0 indicating that the antibody would not effectively bind the antigen at pH 4.0.

The data in Table 2 and Fig. 2 on the other hand, show that the binding of HGH by antibody D was essentially insensitive to changes in pH over the range pH 7.0-10.5 whereas the binding of HGH by antibody C was significantly decreased in the range of pH 9.5-10.5 indicating that the antibody would not effectively bind the antigen at pH 10.5.

The supernatants eluted from antibodies A and C at pH 4.0 and 10.5 were added to PBS buffer (10% in horse serum) and adjusted to pH 7 and the samples pooled. The pooled samples were incubated with polystyrene balls coated with antibodies A, B, C and D and two other monoclonal antibodies against HGH. Each of these antibodies recognize different areas of the HGH molecule. The immunoreactivity of the antigen eluted at either pH 4 or pH 10.5 with five

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of the six antibodies, including antibodies A, B, C and D, had not been affected and was only slightly diminished against the sixth. These data indicate that elution of the antigen at either pH 4.0 or pH 10.5 did not adversely affect its immunochemical properties.

Example 2

A high affinity monoclonal antibody (Ka = 5 x 10^{10}) against prostatic acid phosphatase (PAP), an extremely labile enzyme, obtained by screening hybridomas producing anti-PAP monoclonal antibodies derived from fusions of spleen cells taken from a Balb/c mouse hyperimmunized with PAP and mouse myeloma cells as described in Example 1 was found to exhibit antigen binding sensitivity in the pH range 6.0-4.0. The antibody was bound to sepharose beads using the CNBr technique at a concentration of 1 mg of antibody per 1 ml of packed sepharose beads and used to purify PAP from seminal fluid as follows. A 170 μl sample of seminal fluid containing 0.912 mg/ml of PAP as determined by an immunoradiometric assay, using a TANDEM™ assay kit for PAP manufactured by Hybritech, Inc., San Diego, Ca., was diluted to 5 mls with acetate buffer (10% sodium acetate in horse serum containing 0.15 M NaCl) to obtain a solution of 31 μg PAP/ml of solution having a pH of 6.

The PAP solution was passed through a column containing 1.5 ml of the sepharose beads at the rate of 1 ml/hour and the column washed with 7.5 mls of the starting buffer. Immunometric assay of the eluant (5 mls of sample and 7.4 ml of wash liquid) demonstrated that 99.3% of the PAP had adsorbed to the column. The PAP was elut d with 0.1 M acetate buffer, pH 4 containing 0.15 M NaCl. Three 1 ml fractions were collected and dialyzed overnight vs. 50 mM citrate, pH 6.0. The PAP content of the pooled and

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dialyzed fractions was determined by immunoradiometric assay to be 54% of the total applied to the column. Purity of the dialyzed material was determined by sodium dodecyl sulfate and Ornstein-Davis PAGE. A single band was observed in each case. Enzymatic activity measurements were done and documented that the purified PAP retained its enzymatic activity.

The retention of 46% of the PAP on the column is likely the result, at least in part, of non-specific binding and the use of a large excess of antibody which results in antigen "trail" from the column. The former can be reduced by pretreating the column with sample under the conditions at which elution will be accomplished followed by extensive washing to remove any material which will elute. The latter can be reduced by lowering the concentration of bound antibody. Finally, sepharose is not an ideal matrix for affinity chromatography because of the heterogeneity of pore size, resulting in diffusion and steric problems.

Example 3

The purification of the antigen associated with chlamydia is complicated because it is difficult to solubilize. However, it can be solubilized in a variety of detergents. Hybridomas which produce monoclonal antibodies against chlamydial antigen, obtained by fusing spleen cells from hyperimmunized Balb/c mice with mouse myeloma cells as described in Example 1, were screened for sensitivity to detergent concentration. The effect of detergents on the binding of four such antibodies is set forth in Table 3. The detergents used were deoxycholate (DOC), sodium dodecyl sulfate (SDS) and octylphenoxypolyethoxyethanol sold as Nonidet P-40 (NP 40). Ehrlich ascites was used as a control.

The data in Table 3 were obtained by coating the antigen on microtiter plates and incubating it with a solution of each of the antibodies in a buffer at the

concentration of the detergent indicated in the table. After incubation, the plate is washed and reacted with polyclonal sheep anti-mouse antibodies labeled with horse radish peroxidase (HRP). Incubations were for 1 hr. at room temperature. The plate is washed again and reacted with a solution of orthophenylenediamine (ODP), a chromagen substrate for HRP. Absorbance in each well was measured at 490 nm and is reported in Table 3.

Table 3

Effects of Detergents on Binding By Anti-Chlamydia Monoclonal Antibodies

Antibody Reaction Mixture	O.D.[1] Ehrlich Ascites	O.D.[1] Antibody 1	O.D.[1] Antibody 2	O.D.[1] Antibody 3	O.D.[1] Antibody 4
Aqueous[2] Buffer	0.00	1.06	1.15	1.02	0.95
2% DOC	0.02	1.10	0.70	0.11	0.25
2%NP-40	0.00	0.20	0.11	0.80	0.06
0.5%DOC	0.03	1.37	1.36	1.22	1.25
0.1%SDS	0.05	1.17	1.20	1.30	1.05

1. O.D. at 490 nm obtained as an average of 2 samples with a standard deviation of 0.05.
2. Aqueous buffer is Autopow tissue culture media with 8% horse serum, 2% fetal calf serum. All detergents used in the experiment were diluted in this buffer.
3. Used as a control.

These data show the effect of different detergents and detergent concentration on the binding of the selected monoclonal antibodies. Antibody No. 1 and Antibody 2 have a relatively high affinity for Chlamydia in aqueous buffer that was not affected by any of the detergents except 2% NP-40. Antibody 3 had a low affinity in 2% DOC, yet retained its high affinity in the other media. Antibody 4 had a low affinity in 2% DOC and 2% NP-40 but a high affinity in the other media.

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In other experiments, the antigen coated microtiter plates were first incubated with detergents in the concentrations shown in Table 3 for 1 hr. and then washed. Thereafter, the antichlamydia antibodies were incubated in the wells followed, after washing, by an incubation with the HRP labeled anti-mouse antibodies. This incubation, after washing, was followed by an incubation with the enzyme substrate. The optical densities measured in each well compared to wells which were pretreated with the aqueous buffer suggested that the antigen was not harmed by the detergents. Accordingly, the monoclonal antibodies could be used for the affinity purification of the Chlamydia antigen by solubilizing the antigen in a detergent compatible with antibody binding and passing the preparation over a column of immobilized antibody to bind the antigen. Subsequently, the antigen is released by eluting the column with another detergent composition in which the antibody does not bind to the antigen.

From the foregoing, it will be clear to those skilled in the art that efficient purifiction of an antigen by means of affinity chromatography using a selected monoclonal antibody as the immunoadsorbent can be accomplished under conditions which do not denature the antig n. Specific applications of this process include its use to purify antigens in samples where they occur naturally and to purify radiolabeled antigen which has degraded upon storage. A particular application is the purification of protein products obtained by recombinant DNA technology. Among such products may be mentioned insulin and human growth hormone. The isolation of complex proteins from serum, for example Factor V or Factor VIII, is possible using the process of this invention.

It is also possible to reverse the process and to purify the monoclonal antibody by using immobilized antigen as an immunoadsorbent. For example, radiolabeled antibody used in an immunoradiometric assay which has degraded as a result of storage can be purified in this

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way. The monoclonal antibody can also be recovered from ascites fluid or culture medium by using the immobilized antigen as an immunoadsorbent.

While we do not wish to be bound by any particular theory, the change in Ka with changes in pH is the likely effect of protonation of histidine residues or deprotonation of lysine or possibly tyrosine or aginine residues in either the antibody, the antigen or both which alters the ability of the antigen and antibody to complex with each other. Specific residues affected may or may not lie within the binding regions of the molecules.

Based upon our discovery that the antibodies produced by an animal's immune response to an antigen include antibodies that vary in their sensitivity to chang s in environment, it is within the scope of our invention to fractionate polyclonal antisera to obtain a mixture of antibodies which behave in a manner similar to the environmentally sensitive monoclonal antibodies of this invention. This fractionation can be accomplished by contacting the immobilized antigen with an excess of the antisera in a first desired environmental condition followed by washing the immunoadsorbent to remove unbound material. This step is followed by contacting the immnoadsorbent with a medium which is the second environment to elute antibodies which are not eluted under the first environmental condition. For example, if one wishes to obtain antibodies which exhibit a high affinity at pH 7, and a low affinity at pH 4, the immobilized antigen is contacted with an excess of antisera at pH 7 and the immobilized antigen washed with a medium at pH 7 to remove antibodies which exhibit a low affinity at pH 7. The immunoadsorbent is then eluted at pH 4 to remove antibodies which exhibit a low affinity at pH 4. The eluant will contain the fraction of antibodies whose binding with the antigen is sensitive to changes in pH over the range pH 7 to pH 4. Similar fractionation can be done with urea and other inhibitors of antigen-antibody binding. The

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resulting populations of antibodies may require further subfractionation to further remove those antibodies which elute due to an intrinsic low affinity rather than a Ka "switch".

It is also within the scope of our invention to employ hybrid monoclonal antibodies having dual sp cificities for affinity purification. A process for obtaining hybrid monoclonal antibodies is described in the concurrently filed application of Martinis et al, "Antibodies Having Dual Specificities, Their Preparation And Uses Therefor", Serial No. --, (Lyon and Lyon Docket 162/98), the disclosure of which is incorporated by reference.

The hybrid monoclonal antibody has two specificities which may be for different antigens. For use in our invention, the hybrid antibody is selected to exhibit pH or other environmental sensitivity in the specificity for the antigen for which it is to be used as an immunoadsorbent in an affinity chromatography. The other specificity exhibited by the hybrid is selected to have a high affinity against a second antigen which is bound to a solid support. When the hybrid antibody is applied to the solid support, it is bound to the support by the second antigen. Of course, the affinity of the hybrid for the second antigen must not be substantially lower in the environmental condition which will permit elution of the first, or target antigen. Preferably, however, the binding of second antigen to the antibody is sensitive to a different environmental condition than that which permits the target antigen to be eluted from the immunoadsorbent. For example, the hybrid antibody may be selected so that the affinity for the target antigen is reduced by a lowering of pH and the affinity for the second antigen reduced by increasing the pH. This permits the hybrid monoclonal antibody to be desorbed readily from a solid support when it is desirable to do so because the

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support has become contaminated by impurities or other reasons which impairs its usefulness.

The environmentally sensitive antibodies of our invention can also be used to store antigens in a solid phase that are unstable in solution. For example, radio-labeled antigen can be bound to the immobilized antibody for storage and desorbed as needed. Desorption can be preceded by washing the immunoadsorbent to remove any products of degration that arose during storage. The reverse process is also possible, i.e., the antigen can be used to store unstable antibody in a solid phase. For example, radiolabeled antibody used in a radioassay can be stored as the antigen: antibody complex and desorbed as required.

The foregoing is a description of the presently preferred embodiments of our invention which is to be limited only by the appended claims.

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Claims:

1. A monoclonal antibody having a high affinity for the antigen against which it is raised in a first environment and a low affinity for the antigen in a second environment, neither environment substantially irreversibly altering the immunochemical properties of the antigen or the antibody.

2. A monoclonal antibody according to Claim 1 wherein the binding constant for the antibody to the antigen in the first environment is about $\geq 10^9$ and the binding constant for the antibody to the antigen in the second environment is about $\leq 10^8$.

3. A monoclonal antibody according to Claim 2 wherein the binding constant in the first environment is $\geq 10^{10}$ and the binding constant in the second environment is $\leq 10^6$.

4. A monoclonal antibody according to Claim 1, 2 or 3 wherein the first environment a liquid medium having a first pH and the second environment is a liquid medium having a second pH.

5. A monoclonal antibody according to Claim 4 wherein the pH of the first environment and the pH of the second environment is within the range of about 4 to about 10.5.

6. A monoclonal antibody according to Claims 1, 2 or 3 wherein the second environment is a solution containing a chaotropic agent.

7. A monoclonal antibody according to Claim 6 wherein the chaotropic agent is selected from the group consisting of urea, guanidin , KSCN, KBr, KI and $MgCl_2$.

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8. A hybrid monoclonal antibody having a dual specificity, one sp cificity being against a first antigen and the second specificity being against a second antigen, th specificity against the first antigen having a high affinity in a first environment and a low affinity in a second environment, neither environment substantially irreversibly altering the immunochemical properties of the first antigen or the antibody and wherein the affinity of the hybrid antibody for the second antigen is not substantially different in said first and second environments.

9. A hybrid monoclonal antibody according to Claim 8 wherein the antibody has a high affinity for the second antigen in the first environment and a low affinity for the second antigen in a third environment.

10. A hybrid monoclonal antibody according to Claim 9 wherein the first environment is a liquid medium having a first pH, the second environment is a liquid medium having a second pH and the third environment is a liquid medium having a third pH.

11. A monoclonal antibody according to Claims 1, 2, 3, 8 or 9 wherein the antibody is bound to a solid support.

12. A monoclonal antibody according to Claim 11 wherein the solid support is selected from the group consisting of Sepharose, polystyrene, glass, nylon, cellulose, polymethyl methacrylate, silica gel, polyacrylamide and nitrocellulose.

13. A process for the purification of an antigen comprising the steps:

a) selecting an antibody having a high affinity for the antigen in a first environment and a low affinity

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for the antigen in a second environment, neither environment substantially irreversibly altering the immunochemical or biological properties of the antigen or antibody;

b) immobilizing the antibody on a solid support;

c) binding the antigen to the antibody in the first environment;

d) separating unbound impurities from the bound antigen; and

e) eluting the antigen in a purified form from the immobilized antibody using, as an eluant, a medium which is the second environment.

14. A process according to Claim 13 wherein the antibody is a monoclonal antibody.

15. A process according to Claims 13 or 14 wherein the binding constant for the antibody to the antigen in the first environment is about $\geq 10^9$ and the binding constant for the antibody to the antigen in the second environment is about $\leq 10^8$.

16. A process according to Claims 13 or 14 wherein the binding constant in the first environment is $\geq 10^{10}$ and the binding constant in the second environment is $\leq 10^6$.

17. A process according to Claims 13 or 14 wherein the first environment is a liquid medium having a first pH and the second environment is a liquid medium having a second pH.

18. A process according to Claim 17 wherein the pH of the first environment and the pH of the second environment are within the range of about 4 to about 10.5.

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19. A process according to Claims 13 or 14 wherein the second environment is a solution containing a cha tropic agent.

20. A process according to Claim 19 wherein the chaotropic agent is selected from the group consisting of urea, guanidine, KSCN, KBr, KI and $MgCl_2$.

21. A process according to Claim 14 wherein the monoclonal antibody is a hybrid monoclonal antibody having a first specificity against the antigen to be purified and a second specificity having a high affinity against a second antigen, the second antigen being immobilized on the solid support and providing the means whereby the antibody is immobilized on the solid support, the affinity of the antibody for the second antigen not being substantially lowered in the second environment.

22. A process according to Claim 21 wherein the antibody has a low affinity for the second antigen in a third environment.

23. A process according to Claim 22 wherein the first environment is a liquid medium having a first pH, the second environment is a liquid having a second pH and the third environment is a third liquid having a third pH.

24. A process according to Claim 13 wherein the antibody is an antiserum fraction.

25. A process for fractionating serum antibodies to obtain a fraction having a high affinity for an antigen in a first environment and a low affinity for the antigen in a second environment, neither environment substantially irreversibly altering the immunochemical properties of the antigen or antibodies, comprising immobilizing the antigen on a solid support, contacting the antigen with th serum

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antibodies in the first environment to bind the antibodies to the antigen and eluting a fraction of the antigens using, as an eluant, a medium which is the second environment, the eluted antibodies having a low affinity for the antigen in the second environment.

26. A process according to Claim 25 wherein the first environment is a liquid medium having a first pH and the second environment is a liquid medium having a second pH.

27. A process according to Claim 26 wherein the pH of the first environment and the pH of the second environment is within the range of about 4 to about 10.5.

28. A process according to Claim 25 wherein the second environment is a solution containing a chaotropic agent.

29. A process for the purification of an antibody having a high affinity for an antigen in a first environment and a low affinity for the antigen in a second environment, neither environment substantially irreversibly altering the immunochemical properties of the antigen or antibody, comprising the steps:

a) immobilizing the antigen on a solid support;

b) binding the antibody to the antigen in the first environment;

c) separating the unbound impurities from the bound antibody; and

d) eluting the antibody in a purified form from the immobilized antigen using, as an eluant, a medium which is the second environment.

30. A process according to Claim 29 wherein the antibody is a monoclonal antibody.

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31. A process according to Claim 29 wherein the first environment is a liquid medium having a first pH and the second environment is a liquid medium having a second pH.

32. A process according to Claim 31 wherein said first pH and said second pH are within the range of about 4 to about 10.5.

33. A process according to Claim 29 wherein the second environment is a solution containing a chaotropic agent.

34. A process according to Claim 33 wherein the chaotropic agent is selected from the group consisting of urea, guanidine, KSCN, KBr, KI and $MgCl_2$.

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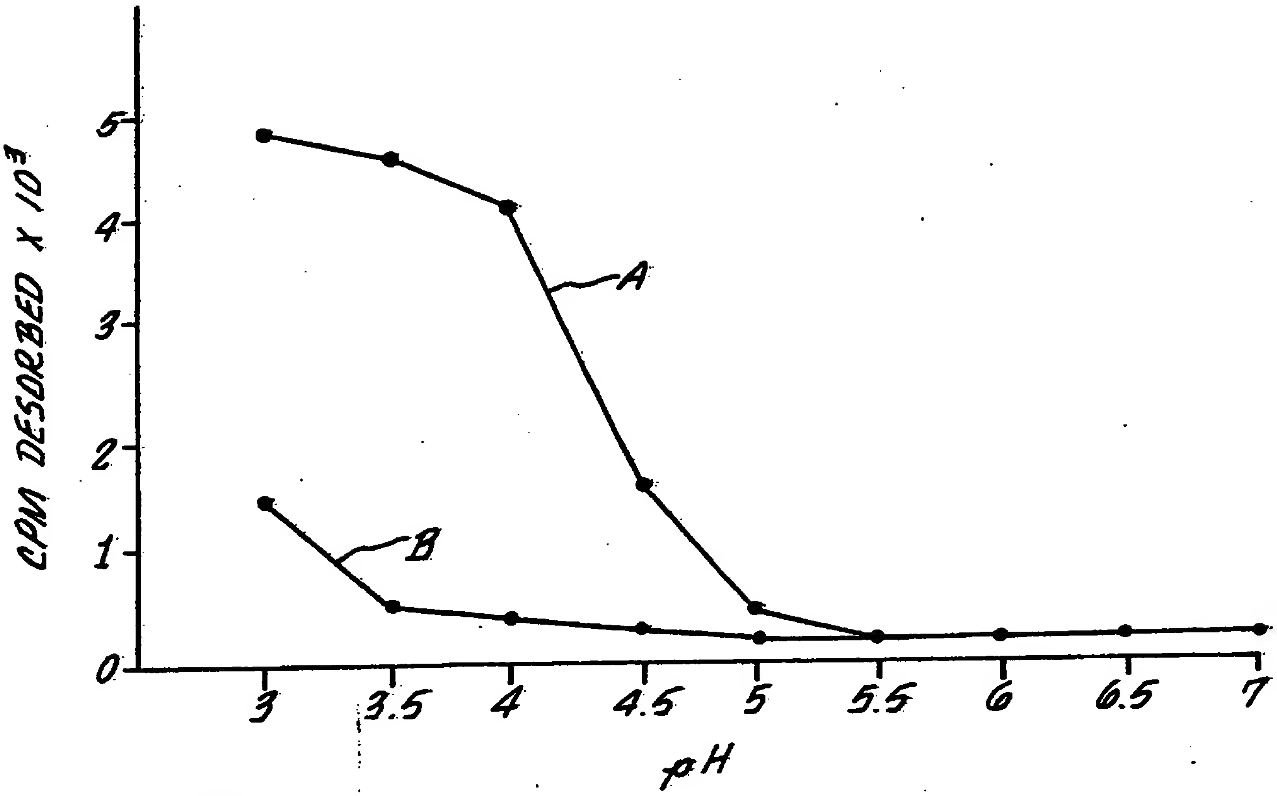

FIG. 1

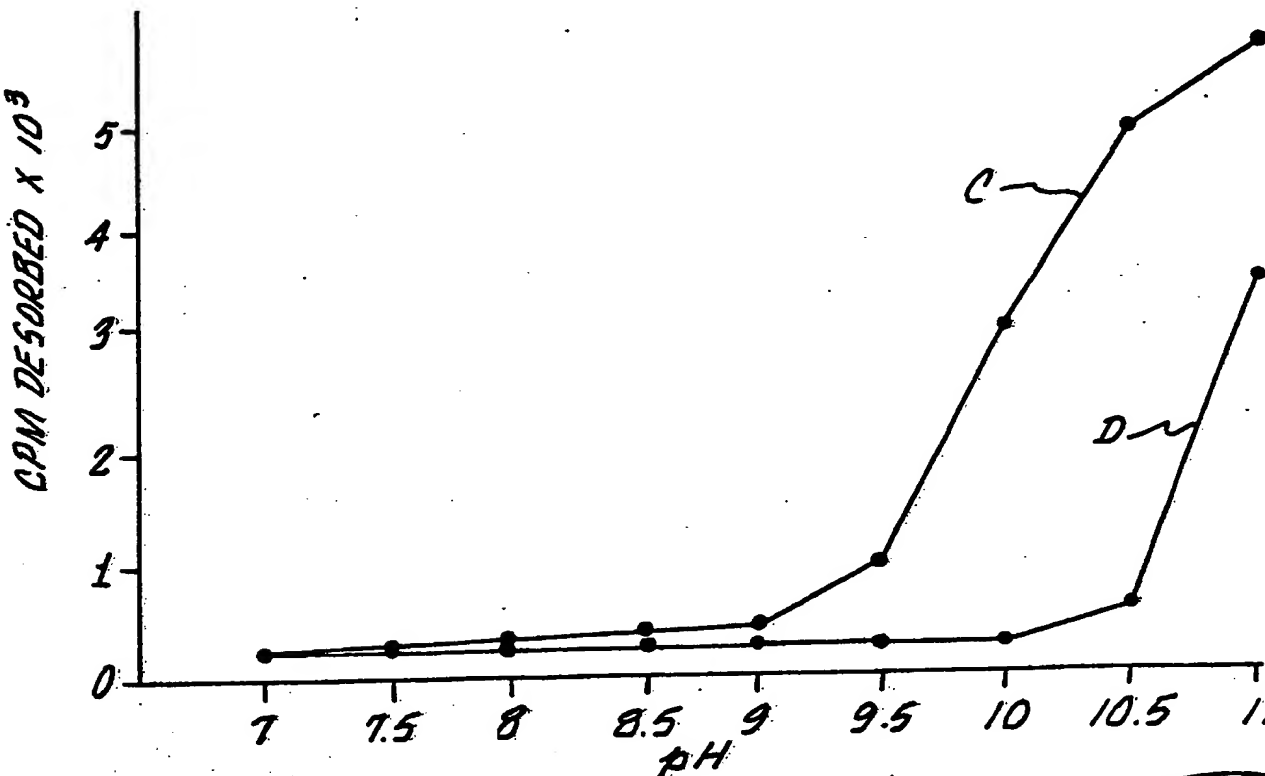

FIG. 2

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INTERNATIONAL SEARCH REPORT

International Application No PCT/US83/00524

I. CLASSIFI ATI N F SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

U.S.CL. 435/68,172, 436/548, 424/85, 260/112B
INT CL 3 G01N 33/54, C12N 15/00, A61K 39/00, C07G 7/00

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	435/68,172,803, 436/548, 424/1,85, 260/112B

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

CHEMICAL ABSTRACTS 1977-1982 ANTIBODY, AFFINITY OR MONOCLONAL

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A	US,A, 4,172,124, 23OCTOBER 1979, KOPROWSKI ET AL.	1-7,13-20
X	N, BEAVO ET AL, "ENZYME PURIFICATION UTILIZING CONFORMATION SPECIFIC MONOCLONAL ANTIBODIES" IN, FELLOWS ET AL, "MONOCLONAL ANTIBODIES IN ENDOCRINE RESEARCH" 1981, RAVEN PRESS, NY, p. 157-166	1-7, 13-20, 24-34
X	N, ROSE ET AL, "PRINCIPLES OF IMMUNOLOGY" 2nd ED., 1979, MACMILLAN PUBLISHING CO., NY, p. 499-501	8-12, 21-23
X	N, JOURNAL OF BIOLOGICAL CHEMISTRY, 256(8), 25APRIL 1981, SPRINGER, "MONOCLONAL ANTIBODY ANALYSIS OF COMPLEX BIOLOGICAL SYSTEMS" p. 3833-3839	1-7, 13-20
X	N, JOURNAL OF IMMUNOLOGY, 127(3), SEPTEMBER 1981, STALLCUP ET AL, "CHARACTERIZATION OF AN ANTI H-2 MONOCLONAL ANTIBODY AND ITS USE IN LARGE SCALE ANTIGEN PURIFICATION" p. 923-30	1-7, 13-20
X	N, JOURNAL OF IMMUNOLOGICAL METHODS, 46(2), 15 OCTOBER 1981, STENMAN ET AL, "CHARACTERIZATION OF A MONOCLONAL ANTIBODY TO HUMAN ALPHAFETOPROTEIN AND ITS USE IN AFFINITY CHROMATOGRAPHY" p. 337-345	1-7, 13-20

* Special categories of cited documents: [15]

"A" document defining the general state of the art
"E" earlier document but published on or after the international filing date
"L" document cited for special reason other than those referred to in the other categories
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but on or after the priority date claimed
"T" later document published on or after the international filing date or priority date and not in conflict with the application, but cited to understand the principle or theory underlying the invention
"X" document of particular relevance

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
22 JULY 1983	02 AUG 1983
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	John E. Tarcza JOHN E. TARCZA

Form PCT/ISA/210 (second sheet) (October 1977)

International Application No. PCT/US83/00524

III. DOCUMENTS C NSIDERED TO BE RELEVANT (NTINUED FR M THE SEC ND SHEET)

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No[18]
X	N, PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES USA, 78(3) MARCH 1981, STAEHELIN ET AL, "PRODUCTION OF HYBRIDOMAS SECRETING MONO-CLONAL ANTIBODIES TO THE HUMAN LEUKOCYTE INTERFERON" p. 1848-1852	1-7, 13-20 24-34
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A	N, CLINICAL CHEMISTRY, 27(11) 1981, SEVIER ET AL, "MONOCLONAL ANTIBODIES IN CLINICAL IM-MUNOLOGY" p. 1797-1806	1-34
A	N, BIOCHEMICAL JOURNAL, 200, 1981, EDWARDS, "SOME PROPERTIES AND APPLICATIONS OF MONO-CLONAL ANTIBODIES" p. 1-10	1-34